Supporting Information

Supercoiling Structure-based Design of a Trimeric Coiled-coil Peptide with High Potency against HIV-1 and Human β-coronavirus Infection

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Supplemental Tables and Figures

Table S1. Summary of the SVA results of N3G alone, N3G/C34 and N3G/HR2P complexes[a]

Peptides	Sedimentation coefficient (s)	Observed molecular mass (kDa)	Calculated molecular mass (kDa)
N3G	0.77	12.8	13.2
N3G/C34 6-HB	2.15	25.3	26.1
N3G/MERS-CoV HR2P 6-HB	2.13	23.2	25.8
N3G/HCoV-OC43 HR2P 6-HB	2.08	23.4	26.2

[a] SVA studies were performed at a concentration of 150 μM in PBS (pH 7.4) and a rotor speed of 60,000 rpm at 20 ℃.

Table S2. Biophysical properties of the designed peptides

Compd	Helicity (%)[a]	*Tm* (℃)[b]
N3G	69.5	>90
N1G	74.7	55.0
N3HR	74.8	>90

[a] CD spectra of each designed peptide were monitored in PBS, pH 7.4. The final concentration of the peptide was 15 μM.
[b] Thermal denaturation CD scans were performed on 15 μM peptide solutions in PBS with 2 M guanidine hydrochloride.

Table S3. HPLC method used for the purification of peptide compounds[a]

Time (min)	Solvent A (%)	Solvent B (%)
5	70	30
13	53	47
30	17	83
35	17	83
55	70	30
60	70	30

[a] The crude peptide products were purified by preparative reverse phase HPLC with a Waters preparative HPLC system (PrepLC 4000) on a Waters X-bridge C8 column (19.5mm × 250mm, 10μm) at constant flow rate of 16 mL/min. Solvent A: 0.1% trifluoroacetic acid in H_2O; Solvent B: 0.1% trifluoroacetic acid in 70% CH_3CN/H_2O.

Table S4. HPLC method used for the analysis of peptide compounds[a]

Methods	Time (min)	Solvent A (%)	Solvent B (%)
Method A	5	40	60
	12	10	90
	20	0	100
	25	90	10
Method B	5	50	50
	10	0	100
	20	0	100
	25	90	10

[a] Peptide compounds were analyzed by analytical RP-HPLC performed on a RP-C8 column (Zorbax Eclipse XDB-C8, 4.6 × 150 mm, 5 μm) using two different solvent systems (Methods A and B) and a flow rate of 1 mL/min with detection at 210 nm. Solvent A: 0.1% trifluoroacetic acid in H_2O; Solvent B: 0.1% trifluoroacetic acid in 70% CH_3CN/H_2O.

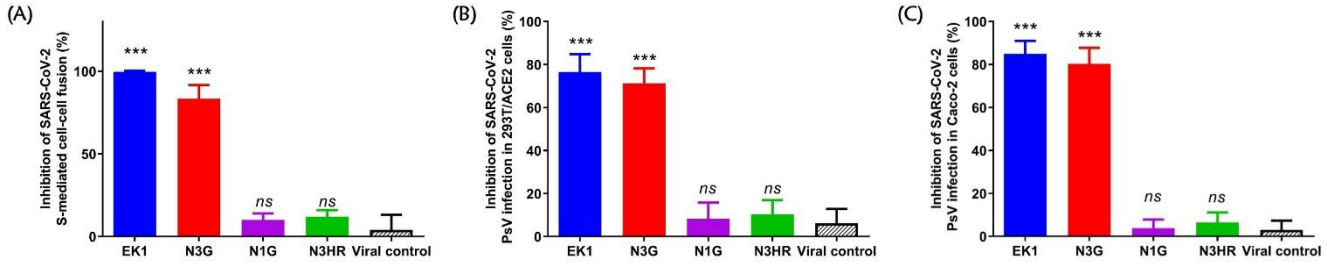


Figure S1. Both N3G and EK1 are effective against SARS-CoV-2 infection. (**A**) Inhibition of peptides on SARS-CoV-2 S-mediated cell–cell fusion at the screening concentration of 20 μM. Inhibition of peptides against pseudotyped SARS-CoV-2 infection in 293T/ACE2 cells (**B**) and Caco-2 cells (**C**). Data are presented as mean ± s.d. of three independent experiments. *** indicates a significant difference from viral control (Student's *t* test, $P < 0.001$). ns: not significant.

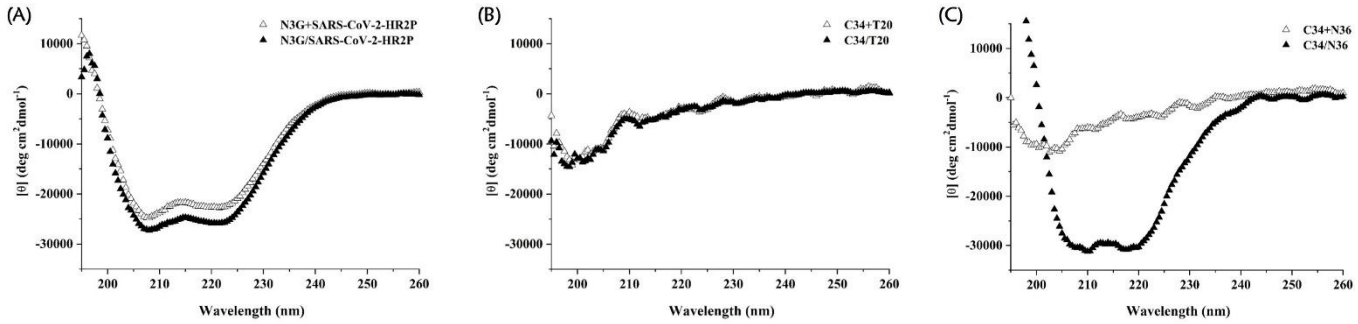


Figure S2. N3G and SARS-CoV-2-HR2P show interaction in solution. (**A**) CD spectrum of SARS-CoV-2-HR2P/N3G mixtures (solid symbol) and the theoretical noninteracting spectra of the related isolated peptides (SARS-CoV-2-HR2P+N3G, open symbol) are shown for comparison. N3G and SARS-CoV-2-HR2P interaction induces more α-helix structure than the sum of the single peptides, which is distinguishable from (**B**) noninteracting T20-C34 peptide pair. (**C**) As a positive control, the interaction between N36 and C34 led to their dramatic secondary structural changes. The final concentration of each peptide in PBS (pH 7.4) was 15 μM.

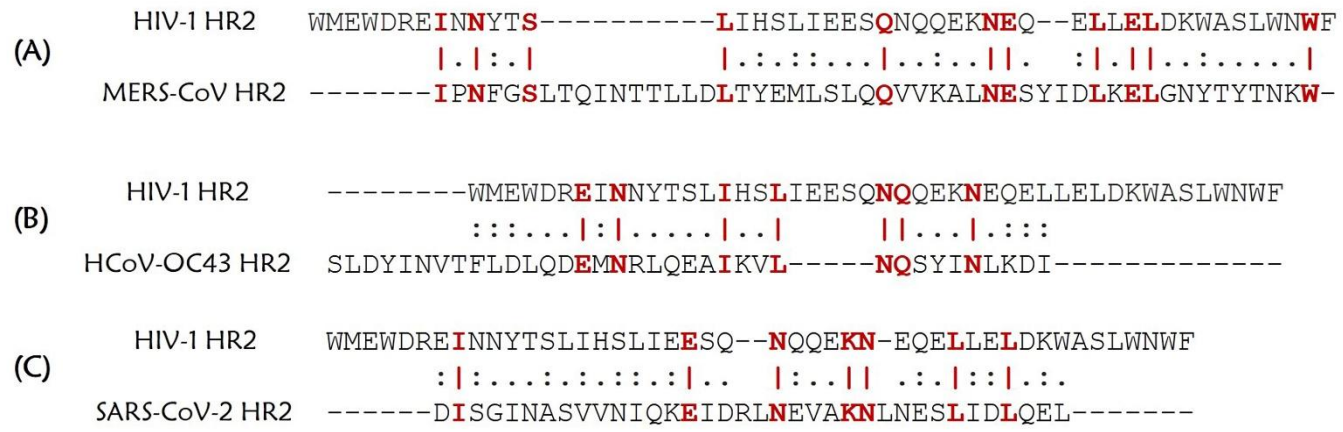


Figure S3. Sequence similarity between the HR2 domain (residues 628–673) in gp41 of HIV-1 and the N-terminal portion of that of (**A**) MERS-CoV (residues 1,246–1,295), (**B**) HCoV-OC43 (residues 1,246–1,295), or (**C**) SARS-CoV-2 (residues 1,168–1,203). Identical amino acid residues are highlighted in red.

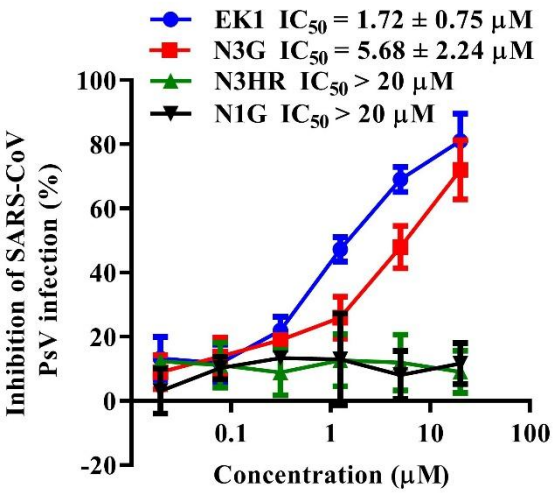
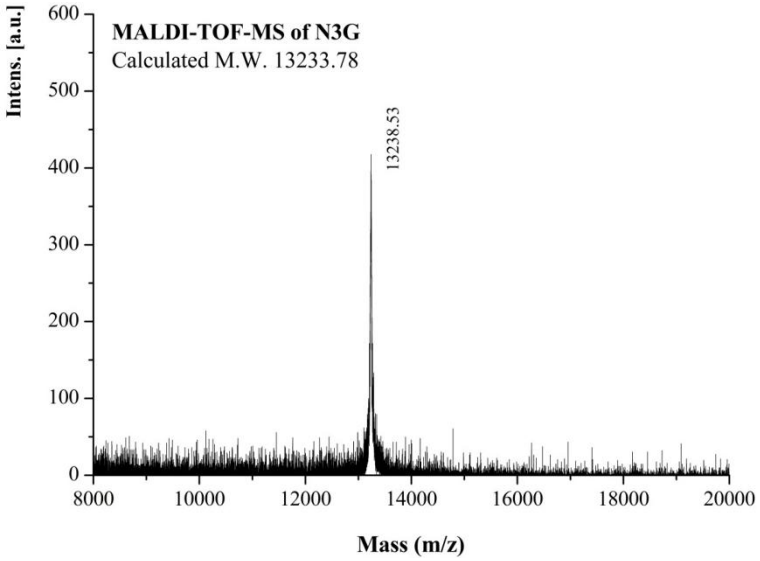
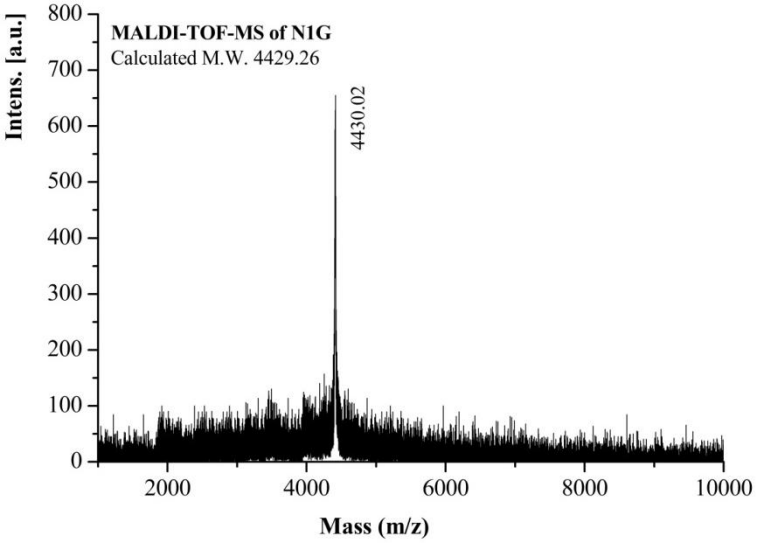
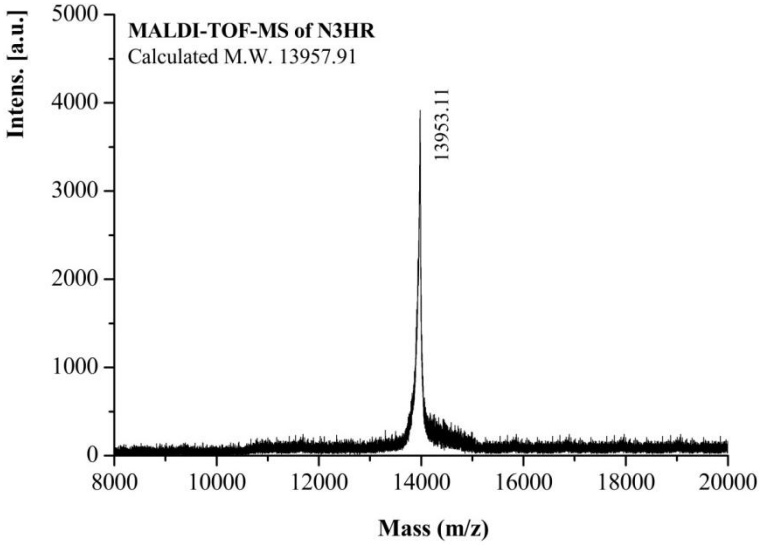


Figure S4. Inhibitory activity of peptides on pseudotyped SARS-CoV infection in Caco-2 cells.

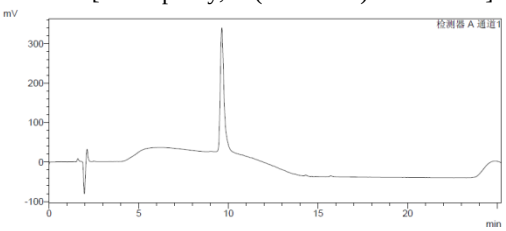
MALDI-TOF-MS and analytical HPLC of designed peptides



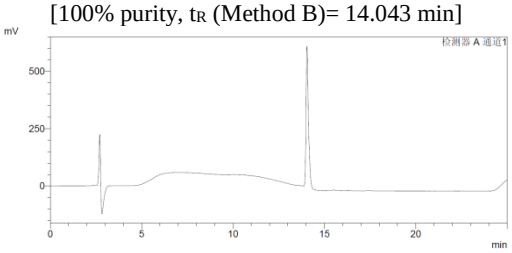




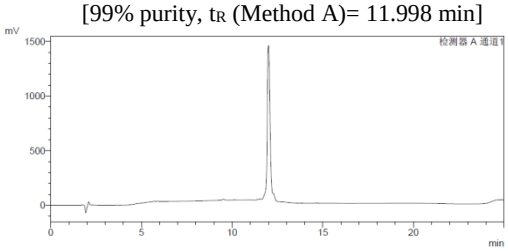
Analytical HPLC of N3G
[100% purity, t_R (Method A)= 9.624 min]



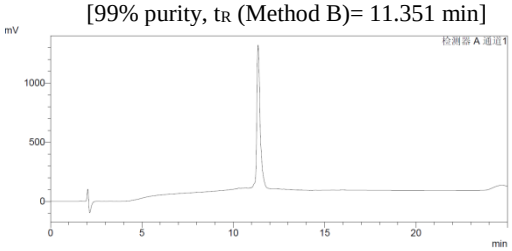
Analytical HPLC of N3G
[100% purity, t_R (Method B)= 14.043 min]



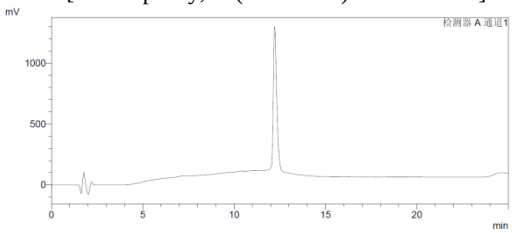
Analytical HPLC of N1G
[99% purity, t_R (Method A)= 11.998 min]



Analytical HPLC of N1G
[99% purity, t_R (Method B)= 11.351 min]



Analytical HPLC of N3HR
[100 % purity, t_R (Method A)= 12.215 min]



Analytical HPLC of N3HR
[98 % purity, t_R (Method B)= 13.518 min]